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Synthesis and influenza virus sialidase inhibitory activity of analogues of 4-guanidino-Neu5Ac2en (GG167) with modified 5-substituents

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Summary — Analogues of 4-guanidino-Neu5Ac2en (GG167) have been prepared containing alternative amide and sulfonamide substituents at the 5-position. (4*S*,5*R*,6*R*)-4-guanidino-5-(2,2,2-trifluoroacetylamino)-6-(1*R*,2*R*,3-trihydroxypropyl)-5,6-dihydro-4*H*-pyran-2-carboxylic acid **5** and (4*S*,5*R*,6*R*)-4-guanidino-5-methanesulfonylamino-6-(1*R*,2*R*,3-trihydroxypropyl)-5,6-dihydro-4*H*-pyran-2-carboxylic acid **6** were the only analogues which approached the activity of GG167, showing potent inhibition of influenza virus sialidases and good antiviral activity in vitro.

influenza virus sialidase neuraminidase GG167 / antiviral activity

Introduction

2,3-Didehydro-2,4-dideoxy-4-guanidinyl-*N*-acetylneuraminic acid **1** (4-guanidino-Neu5Ac2en, GG167) is a potent and highly selective inhibitor of influenza virus sialidase which is currently undergoing clinical evaluation for the treatment of influenza [1]. GG167 was discovered by rational drug design [2, 3]. It was initially conceived by applying molecular modelling and computational chemistry techniques to the X-ray crystal structure of sialic acid bound to influenza sialidase. In several recent reports [4–6, 8] we have described structural modifications to GG167. These studies were carried out in order to establish the contribution of each of the dihydropyran substituents to influenza sialidase affinity (fig 1). For example, deletion of the glycerol sidechain demonstrated the very important contribution to the overall binding of GG167 made by the 8,9-diol moiety (which forms hydrogen bonds to a conserved acidic residue in the sialidase) [4]; and modifications at the 4-position of the dihydropyran ring (the position occupied by the guanidino group in GG167) established that a small basic group at this position is required for optimum binding and selectivity [5, 6]. Thus, the simple amine 2,3-didehydro-2,4-dideoxy-4-amino-*N*-acetylneuraminic acid **2** is also a potent inhibitor of influenza virus sialidase.

A further substituent on the dihydropyran which is available for modification is the 5-acetamide. In early work in this area Meindl and Tuppy [7] modified this

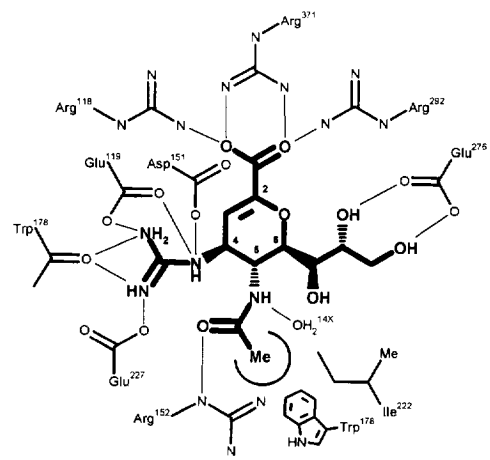

Fig 1. Schematic representation of non-covalent interactions between GG167 and influenza A sialidase.

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substituent in the related compound 2,3-didehydro-2-deoxy-*N*-acetylneuraminic acid (Neu5Ac2en, DANA, **3**). They prepared a number of alternative amides, and found that the trifluoroacetamide, 2,3-didehydro-2-deoxy-*N*-trifluoroacetyl neuraminic acid (FANA, **4**), was the most potent influenza sialidase inhibitor of the series. (The measured K_i values for FANA **4** against several influenza sialidases were four to five fold lower those of DANA **3**.) In a previous communication we showed that completely removing the 5-acetamido substituent from GG167 produced a dramatic loss of enzyme inhibitor activity [8]. Herein we describe the synthesis and influenza virus sialidase inhibition of a series of 5-amides **5**, **7–11** and sulfonamides **6**, **12–14** (fig 2) which are analogues of **1** and **2**.

Chemistry

The syntheses of compounds **5–14** are outlined in schemes 1–4. Initially methyl ester **15** [9] was deacetylated using a modification of the procedures of Grieco and Ragnarsson (scheme 1) [10, 11]. Thus treatment of **15** over several hours with portions of di-*tert*-butyldicarbonate and 4-dimethylamino pyradine (DMAP) in dioxane afforded a good yield of the 5-*N*-Boc derivative **16**. It is noteworthy that the use of DMAP is essential for this conversion to occur, and that the yield of **16** is significantly higher in dioxane than in either dichloromethane or acetonitrile. Treatment of **16** with sodium methoxide in methanol cleanly removed the 5-*N*-acetyl group and also the

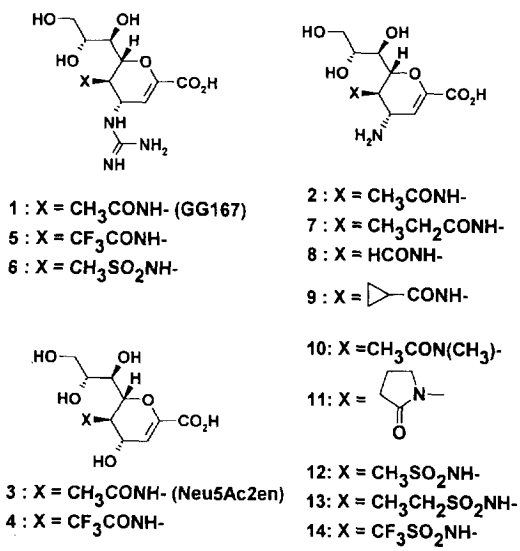
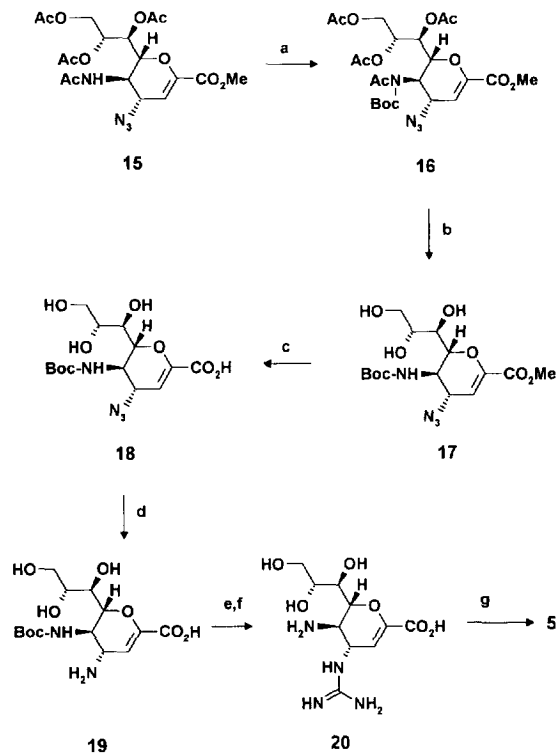


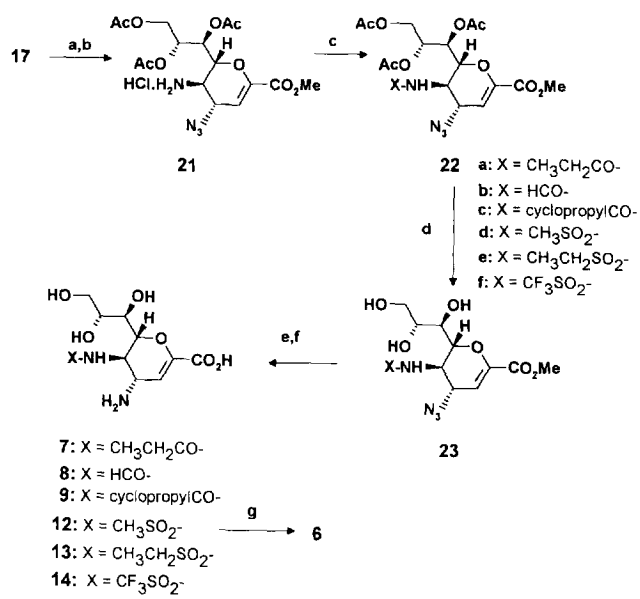
Fig 2. Structure of compounds **1–14**.



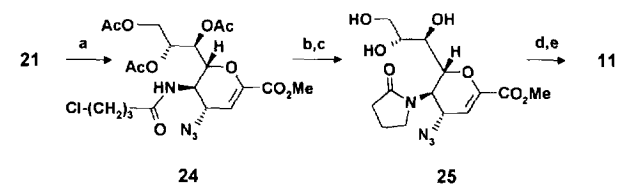
Scheme 1. (a) $(t\text{-BuOCO})_2O$, DMAP, dioxane (86%); (b) NaOMe, MeOH (70%); (c) NaOH aq (66%); (d) Ph_3P, MeOH (45%); (e) AIMSA; (f) CF_2CO_2H (13%, **19** → **20**); (g) CF_2CO_2Me, Et_3N (30%, **20** → **5**).

three *O*-acetates affording 4-azido-Neu5Boc2en methyl ester **17**. For the synthesis of the target *N*-trifluoroacetyl compound 5 (scheme 1), the methyl ester was next hydrolysed with aqueous sodium hydroxide. This gave 4-azido-Neu5Boc2en **18** after ion exchange chromatography. Reduction of the azide group in **18** with triphenylphosphine afforded amine **19**. Guanylation of the 4-amino group in **19** with aminoiminomethanesulfonic acid (AIMSA) [11] produced 4-guanidino-Neu5Boc2en from which the Boc group was removed by treatment with trifluoroacetic acid (TFA), yielding **20**. The synthesis of **5** was completed by selective trifluoroacetylation of **20** with methyl trifluoroacetate.

By modifying the order in which the above transformations were carried out, an alternative route from **17** (scheme 2) was also devised which enabled the preparation of the 5-modified derivatives **6–14**. Thus *O*-acetylation of **17** with acetic anhydride in pyridine and removal of the 5-*N*-Boc protection with HCl in dioxane afforded intermediate **21**. This was treated with a range of acylating and sulfonylating reagents to form the intermediates **22a–f**. Hydrolysis



Scheme 2. (a) Ac_2O, pyridine (98%); (b) HCl/dioxane (99%); (c) acylation with X-Cl; (d) NaOMe, MeOH; (e) Ph_3P; (f) Et_3N aq; (g) AIMSA (18%).

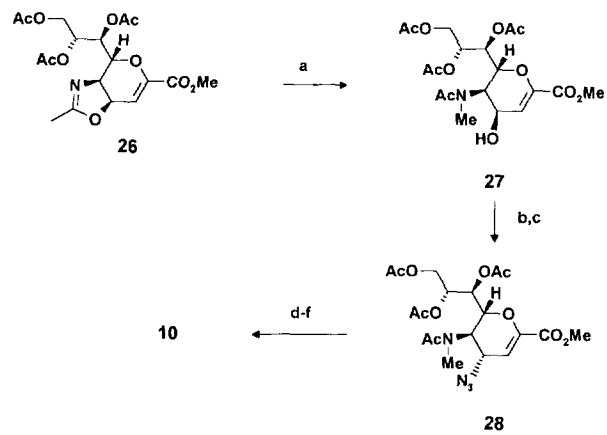


Scheme 3. (a) $Cl(CH_2)_3COCl$ (66%); (b) KI, K_2CO_3, acetone (42%); (c) NaOMe/MeOH (55%); (d) Ph_3P; (e) aq Et_3N (45%, **25** → **11**).

of the *O*-acetates in these compounds with sodium methoxide in methanol produced the triols **23a–f**. Finally, triphenylphosphine reduction of the azide groups in **23a–f** and hydrolysis of the methyl esters with aqueous triethylamine afforded the target amides **7–9** and sulfonamides **12–14**. The methanesulfonamide **12** was converted into the corresponding 4-guanidino target **6** by treatment with AIMSA.

Cyclic lactam **11** was prepared via acylation of **21** with chlorobutyryl chloride (scheme 3). This produced the chlorobutyramide **24**. Cyclization of this intermediate with potassium iodide and base, then removal of the *O*-acetates afforded the lactam **25**, which was elaborated to the target **11** using the one-pot, two-step reduction–hydrolysis sequence described above.

The 5-*N*-methyl analogue **10** was prepared from the oxazoline **26** [9, 13] as shown in scheme 4.



Scheme 4. (a) $Me_3O^+BF_4^-$ (69%); (b) $(CF_3SO_2)_2O$; (c) NaN_3 (72%, **27** → **28**); (d) NaOMe/MeOH (85%); (e) Ph_3P; (f) aq Et_3N (45%).

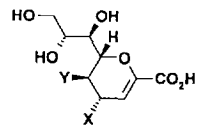
Alkylation with trimethyl oxonium tetrafluoroborate [14] produced the 4-β-hydroxy intermediate **27**. The 4-hydroxyl group was converted into the trifluoromethanesulfonate and then displaced by sodium azide with inversion of configuration, to afford **28**. This compound was elaborated into **10** by the standard reduction and hydrolysis procedure.

Results and discussion

New compounds **5–14** were evaluated for their ability to inhibit the hydrolysis of 2'-(4-methylumbelliferyl)-α-D-*N*-acetylneuraminic acid by sialidases from representative strains of influenza A and B viruses by the previously reported method [15, 16]. The more active inhibitors which emerged from this assay were further evaluated for their in vitro antiviral activity in a plaque reduction assay using MDCK cells [16, 17]. The results obtained are shown in table I together with values for the known inhibitors **1–3**.

The most significant finding in these results is that the trifluoroacetyl analogue **5** has useful biological activity which approaches that of GG167 **1**. Overall, however, its measured enzyme inhibition and antiviral activity is four to five times lower than that of GG167 **1**. Indeed, none of the other substituents introduced at the 5-position in this study produced an inhibitor with biological activity which was as good as the corresponding parent 5-acetamido derivatives **1** (GG167, 4-guanidino) or **2** (4-amino). Increasing or decreasing the size of the alkyl group on the amide substituent by one carbon atom (**7**, **8** vs **2**) resulted in a 10–100-fold reduction in activity, and when the size of the alkyl substituent was increased further, all inhibitory activity was lost (compound **9**). These results are consistent with the X-ray analysis of GG167

Table I. Influenza sialidase inhibition and virus plaque reduction by compounds **1–14**[a].



Compound	*X*	*Y*	*Flu A Aichi*		*Flu B Victoria*	
			Enzyme IC_{50} *(μM)*	*Plaque* IC_{50} *(μg/mL)*	*Enzyme* IC_{50} *(μM)*	*Plaque* IC_{50} *(μg/mL)*
1	$NHC(=NH)NH_2$	CH_3CONH-	0.005	0.005	0.004	0.002
2	NH_2	CH_3CONH-	0.32	0.47	0.41	0.02
3	OH	CH_3CONH-	8.6	24	15	12
5	$NHC(=NH)NH_2$	CF_3CONH-	0.021	0.03	0.024	0.01
6	$NHC(=NH)NH_2$	CH_3SO_2NH-	0.086	0.085	1.1	0.09
7	NH_2	CH_3CH_2CONH-	4.3	14.5	7.2	0.59
8	NH_2	HCONH-	31	16	–	8.4
9	NH_2		> 430	–	> 430	–
10	NH_2	$CH_3CON(Me)$-	540	170	–	–
11	NH_2		> 460	–	> 460	–
12	NH_2	CH_3SO_2NH-	1.7	0.19	3.8	0.09
13	NH_2	$CH_3CH_2SO_2NH$-	210	22	–	5
14	NH_2	CF_3SO_2NH-	> 340	–	–	–

[a]*Sialidase assay:* the IC_{50} values are calculated from the percent inhibition of enzyme activity in the presence of inhibitor relative to a positive (no inhibitor) control. All reactions were carried out in triplicate, and the mean values of these replicates used in the analysis of data. *Plaque assay:* the percent inhibition of plaque formation relative to controls was calculated for each inhibitor concentration used. At each concentration, data from three experiments were pooled in order to accurately determine the 50% inhibitory concentration (IC_{50}) for each compound. In most cases corresponding IC_{50}s from different experiments differed by a factor of no more than 2 to 5.

bound to influenza A sialidase [2]. In the crystal structure, the methyl group of the 5-acetamide of GG167 appears to make hydrophobic contacts with Trp 178 and Ile 222, and there appears to be little further space available to accommodate a larger group. However, inspection suggests that space may be available to accommodate a small substituent on the nitrogen atom of the acetamide. In the enzyme-bound conformation of GG167, this space is occupied by an intervening water molecule (H_2O 14X, fig 1). However, from the poor activities observed for the *N*-methyl compound **10** and the cyclic analogue **11** we conclude that this water is not readily displaced from the enzyme/inhibitor complex.

A further interesting compound to emerge from this study is the methanesulfonamide **6**. Replacement of the acetamide with a methanesulfonyl group results in no appreciable loss of sialidase inhibitory activity (compare **12** with **2**, and **6** with **1**). Once again, however, increasing the size of the alkyl substituent (compound **13**) reduced activity significantly. The inactivity of the trifluoromethanesulfonamide **14** may be related to the high acidity of the sulfonamide NH. It will be deprotonated at physiological pH, and this may produce an unfavourable interaction with the enzyme.

Conclusion

We have described the preparation and biological properties of compound **5**, which is the 5-trifluoroacetamido analogue of GG167 **1**. Compound **5** was shown to be a potent inhibitor of influenza virus sialidase although its measured activity was slightly lower than that of GG167. It also displayed useful, but slightly reduced antiviral activity in vitro. A further selection of 5-modified derivatives were also prepared in order to investigate the structural requirements at this position for optimal enzyme affinity. None of the analogues that we prepared had improved biological activity over GG167, but in addition to **5** the methanesulfonamide **6** also displayed a useful level of sialidase inhibition and antiviral activity in vitro.

Experimental protocols

General

FTIR spectra were recorded using a Nicolet 20SXB or a Bio-Rad FTS-7. ^{1}H-NMR spectra were recorded either at 250 MHz using a Bruker AC or AM 250 or at 400 MHz with a Varian VXR 400. Mass spectra were measured on a HP Engine (Thermospray positive) or VG Autospec Q (LSIMS). Routine microanalyses were performed on a Leco CHNS-932 or Carlo-Erba instrument. Water analyses were performed using a Mitsubishi CA-05. Flash chromatography was performed with a Merck Kieselgel 9385. Analytical HPLC was performed using a Rainin C18 8 μM column, eluting with 0.1% aqueous trifluoroacetic acid at a flow rate of 1 mL per min.

Syntheses

(4S,5R,6R)-5-(Acetoxy-tert-butoxycarbonyl-amino)-4-azido-6-(1S,2R,3-triacetoxy-propyl)-5,6-dihydro-4H-pyran-2-carboxylic acid methyl ester ***16***

To a solution of **15** (10 g, 21.9 mmol) in 1,4-dioxane (100 mL) was added di-*tert*-butyl dicarbonate (Boc_2O) (9.55 g, 43.8 mmol) and 4-dimethylamino pyridine (500 mg, catalytic). After 72 h, TLC analysis showed a clean but slow conversion of **15** to **16**. The solvent volume was reduced by approximately two thirds and a second portion of Boc_2O (3 g) added. The reaction was stirred for 17 h, and then a third batch of Boc_2O (3 g) added and the reaction heated at 80 °C for 2 h until all the starting material had been consumed. The solvent was evaporated and the resulting black oil purified by flash chromatography (eluant ethyl acetate/cyclohexane, 2:3) to yield **16** as an orange oil (10.46 g, 86%): IR (KBr) υ_{max} 2099, 1746, 1690, 1371, 1233 cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 5.99 (br s, 1 H, H-3), 5.27–5.15 (m, 2 H, H-7, H-8), 4.95–4.65 (m, 3 H, H-9, H-6, H-4), 4.5 (m, 1 H, H-5), 4.15–4.02 (m, 1 H, H-9), 3.75 (s, 3 H, $COOCH_3$), 2.35 (s, 3 H, $NHCOCH_3$), 1.99 (s, 9 H, 3 x $OCOCH_3$), 1.55 (s, 9 H, *t*-Bu); MS 574 $(M + NH_4)^+$, 474 $(MNH_4$-*t*Boc$)^+$; high resolution MS: found 574.2352, calc for $C_{23}H_{36}N_5O_{12}$ 574.2360 (error 1.4 ppm).

(4S,5R,6R)-4-Azido-5-tert-butoxycarbonyl-amino-6-(1R,2R,3-trihydroxy-propyl)-5,6-dihydro-4H-pyran-2-carboxylic acid methyl ester ***17***

A solution of **16** (5.05 g 9.1 mmol) in methanol (30 mL) was treated with sodium methoxide (30% w/v solution in methanol 260 μL) under nitrogen at room temperature. After 60 min more methanol (10 mL) was added to dissipate the forming precipitate. After 17 h the solvent was removed in vacuo to give a brown solid which was further stirred with both diethyl ether (100 mL) and water (100 mL) for 15 min. The completely insoluble white precipitate which formed was collected by filtration, washed with further ether and dried in vacuo (60 °C) to give **17** (2.48 g, 70%) as white crystals: mp 191 °C; IR (Nujol) υ_{max} 3358, 3482 3442, 2096, 1734, 1685 cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 7.23 (d, 1 H, NH, J = 9 Hz), 5.79 (d, 1 H, H-3, J = 2 Hz), 4.65 (d, 1 H, OH, J = 5 Hz), 4.49–4.34 (m, 3 H, includes 2 x OH), 4.15 (d, 1 H, J = 11 Hz), 3.75–3.54 (m, 3 H), 3.52–3.37 (m, 2 H), 3.72 (s, 3 H, $COOCH_3$), 1.40 (s, 9 H, *t*-Bu); MS 411 $(M + Na)^+$, 406 $(M + NH_4)^+$; anal $C_{15}H_{24}N_4O_8$ (C, H, N).

(4S,5R,6R)-4-Azido-5-tert-butoxycarbonyl-amino-6-(1R,2R,3-trihydroxy-propyl)-5,6-dihydro-4H-pyran-2-carboxylic acid ***18***

Azide **16** (0.18 g, 0.46 mmol) was dissolved in anhydrous methanol (25 mL) containing sodium methoxide (26 mg, 0.58 mmol). The mixture was stirred at rt for 3 h before it was evaporated to dryness. The resulting residue containing **17** was stirred in 0.1 M sodium hydroxide solution (10 mL) at rt for 4 h. The solution was then adjusted to pH 7 with Dowex-50W X 8 (H^+) resin and filtered. The filtrate was evaporated to dryness to afford **18** (0.12 g, 66%) as a light brown solid: IR (KBr) υ_{max} 3400 (br), 2980, 2930, 2100 (N_3), 1690, 1600 (br) cm^{-1}; ^{1}H NMR (D_2O): δ 5.60 (br s, 1 H, H-3), 4.22 (d, 1 H, H-6, J = 8.2 Hz), 4.17 (d, 1 H, H-4, J = 11.1 Hz), 3.92–3.7 (m, 3 H, H-5, 8, 9), 3.65 (d, 1 H, H-7, J = 9.3 Hz), 3.56 (dd, 1 H, H-9', J = 6.2, 12.1 Hz), 1.36 (s, 9 H, *t*-Bu); MS 397 $(M + H)^+$.

(4S,5R,6R)-4-Amino-5-tert-butoxycarbonyl-amino-6-(1R,2R,3-trihydroxy-propyl)-5,6-dihydro-4H-pyran-2-carboxylic acid ***19***

To a solution of **18** (0.12 g, 0.3 mmol) in a mixture of DMF (6 mL) and pyridine (12 mL) was added triphenylphosphine (0.16 g, 0.6 mmol) at rt. The mixture was stirred under argon at rt for 2 h, and then evaporated under high vacuum to dryness. The residue was stirred in methanol (10 mL) at rt for 1 h, and then evaporated to dryness. The residue was chromatographed (eluant, ethyl acetate/propan-2-ol/water 10:2:1 to 4:2:1) to afford **19** as an off-white solid (0.05 g, 45%). IR (KBr) υ_{max} 3400 (br), 3000–2900, 1710, 1610 (br) cm^{-1}; ^{1}H-NMR (D_2O) δ: 5.60 (br s, 1 H, H-3), 3.98 (br d, 1 H, H-6, J = 9.4 Hz), 3.86 (br d, 1 H, H-4, J = 9.2 Hz), 3.82–3.7 (m, 3 H, H-5, 8, 9), 3.56 (br dd, 1 H, H-9, J = 5.6, 12.4 Hz), 3.51 (br d, 1 H, H-7, J = 9.2 Hz), 1.31 (s, 9 H, *t*-Bu); MS 371 $(M + H)^+$.

(4S,5R,6R)-5-Amino-4-guanidino-6-(1R,2R,3-trihydroxy-propyl)-5,6-dihydro-4H-pyran-2-carboxylic acid ***20***

To a well-stirred solution of the amino acid **19** (50 mg, 0.135 mmol) in water (5 mL) were added AIMSA (0.17 g, 1.23 mmol) and potassium carbonate (0.194 g, 1.95 mmol) over a period of 8 h at 35–40 °C. The mixture was allowed to stand at rt overnight, then diluted with water (10 mL) and filtered. The filtrate was neutralised with 1 M HCl to pH 6, and then evaporated in vacuo. The residue was stirred in trifluoroacetic acid (2 mL) at rt for 2 h then concentrated. The resulting residue was partitioned between water (10 mL) and ethyl acetate (10 mL). The aqueous layer was washed with further ethyl acetate (5 mL) and then passed through a column of Amberlite IR-120 (H^+) resin (10 mL). The column was washed with water (30 mL), and then the resin eluted with a 0.2–1.0 M gradient of ammonium hydroxide solution. The eluate, which was positive towards both ninhydrin and Sakaguchi reagents, was collected, evaporated to dryness, and then freeze-dried to afford 4-guanidino-Neu2en **20** as a white solid (5 mg, 13%). IR (KBr): υ_{max} 3370 (br), 1680, 1600 (br), 1410, 1090 cm^{-1}. ^{1}H-NMR (D_2O): δ 5.46 (d, 1 H, H-3, J = 1.9 Hz), 4.10 (dd, 1 H, H-4, J = 1.9, 9.6 Hz), 3.94 (br d, 1 H, H-6, J = 10.6 Hz), 3.87 (m, 1 H, H-8), 3.82 (dd, 1 H, H-9, J = 2.6, 11.7 Hz), 3.80 (br d, 1 H, H-7, J = 9.5 Hz), 3.61 (dd, 1 H, H-9', J = 5.8, 11.7 Hz), 2.96 (dd, 1 H, H-5, J = 9.6, 10.6 Hz); ^{13}C-NMR (D_2O): δ 173.7 (C-1), 162.3 (C-10), 153.9 (C-2), 108.7 (C-3), 82.6 (C-6), 70.4 (C-8), 67.4 (C-9), 56.9 (C-4), 52.7 (C-5); MS 291 $(M + H)^+$.

(4S,5R,6R)-4-Guanidino-5-(2,2,2-trifluoro-acetylamino)-6-(1R,2R,3-trihydroxy-propyl)-5,6-dihydro-4H-pyran-2-carboxylic acid ***5***

To a stirred solution of **20** (50 mg, 0.17 mmol) in methanol (50 mL) were added methyl trifluoroacetate (0.5 mL, mmol) and triethylamine (0.28 mL, mmol) at rt over a period of 8 h. The resulting solution was stirred at rt for 72 h, and then evaporated to dryness. The residue was purified by flash chromatography (eluant, 2-propanol/water, 3:1). The fractions of R_f = 0.7 were collected and evaporated to dryness. The residue was treated with propanol/water 95:5 solution (10 mL) to precipitate the title compound **5** as a white solid (20 mg, 30%): IR (KBr): υ_{max} 3470 (br), 1710, 1650 (br), 1430, 1220 cm^{-1};

^{1}H-NMR (D_2O) δ 5.53 (d ,1 H, H-3, *J* = 1.6 Hz), 4.49 (dd, 1 H, H-4, *J* = 1.6, 9.4 Hz), 4.39 (br d, 1 H, H-6, *J* = 10.6 Hz), 4.25 (dd, 1 H, H-5, *J* = 9.4, 10.6 Hz), 3.85 (ddd, 1 H, H-8, *J* = 2.3, 6.3, 9.2 Hz), 3.79 (dd, 1 H, H-9, *J* = 2.3, 11.9 Hz), 3.54 (dd, 1 H, H-9', *J* = 6.3, 11.9 Hz), 3.52 (br d, 1 H, H-7, *J* = 9.2 Hz); ^{13}C-NMR (D_2O) δ 168.8 (C-1), 157.1 (C-10), 151.2, 149.5 (C-2, 11), 103.6 (C-3), 74.9 (C-6), 69.8 (C-8), 68.1 (C-7), 63.0 (C-9), 50.8 (C-4), 48.7 (C-5); MS 387 $(M + H)^+$, anal $C_{12}H_{17}F_3O_7N_4 \cdot 3H_2O$ (C, H, N).

(4S,5R,6R)-5-Amino-4-azido-6-(1S,2R,3 triacetoxypropyl)-5,6-dihydro-4H-pyran-2-carboxylic acid methyl ester hydrochloride salt ***21***

A solution of **17** (5.22 g, 13.5 mmol) in acetic anhydride (50 mL) and pyridine (50 mL) was stirred for 18 h by which time all the starting material was consumed. The solution was reduced to an oil by evaporation in vacuo. This oil was taken up in ethyl acetate (100 mL), washed with water (100 mL) and brine, dried over $MgSO_4$ and evaporated in vacuo to an orange gum. The gum was coevaporated with ether to give the triacetate as a white foam (6.84 g, 98%). Triacetate: IR (KBr) υ_{max} 2098 (N_3), 1745, 1721 (C=O) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 7.18 (d, 1 H, NH, *J* = 10 Hz), 5.83 (d, 1 H, H-3, *J* = 2 Hz), 5.36 (dd, 1 H, H-7, *J* = 6, 1 Hz), 5.23 (m, 1 H, H-8), 4.45 (dd, 1 H, H-9, *J* = 12, 2 Hz), 4.32 (dd, 1 H, H-4, *J* = 9, 2 Hz,), 4.22 (dd, 1 H, H-6, *J* = 1, 10 Hz), 4.07 (dd, 1 H, H-9, *J* = 7, 12 Hz), 3.74 (m, 1 H, H-5), 3.72 (s, 3H, CO_2CH_3), 2.03 (s, 3 H, $OCOCH_3$), 1.98 (s, 6 H, 2 x $OCOCH_3$), 1.37 (s, 9 H, *t*-Bu); MS 532 $(M + NH_4^+)$; anal $C_{21}H_{30}N_4O_{11}$ (C, H, N).

A stirred solution of the triacetate (3 g, 5.8 mmol) in 1,4-dioxane (5 mL) was treated with 4 M HCl/dioxane (10 mL). After 3 h the solvent was removed to yield **21** as a beige foam (2.6 g, 99%): IR (KBr) υ_{max} 2108, 1740 (C=O) cm^{-1}. ^{1}H-NMR (DMSO-d_6): δ 8.74 (br s, 3 H, NH_3^+), 6.29 (d, 1 H, H-3, *J* = 4 Hz), 5.45–5.33 (m, 2 H, H-7, H-8), 4.68 (dd, 1 H, H-9, *J* = 3, 7 Hz), 4.63 (t, 3 Hz, 1 H, H-4), 4.39 (dd, 1 H, H-6, *J* = 12, 2 Hz), 4.20 (dd, 1 H, H-9, *J* = 12, 7 Hz), 3.75 (m, 1 H, H-5), 3.74 (s, 3 H, CO_2CH_3), 2.07 (s, 3 H, $OCOCH_3$), 2.03 (s, 3 H, $OCOCH_3$), 2.01 (s, 3H, $OCOCH_3$).

(4S,5R,6R)-4-Azido-5-propionylamino-6-(1S,2R,3-triacetoxypropyl)-5,6-dihydro-4H-pyran-2-carboxylic acid methyl ester ***22a***

A solution of **21** (250 mg, 0.56 mmol) in anhydrous dichloromethane (1.5 mL) was treated under nitrogen with triethylamine (0.36 mL) and propionyl chloride (0.075 ml, 1.5 equiv) at 0 °C. After 2 h the organic solution was partitioned between 2 N HCl (50 mL) and ethyl acetate (50 mL). The ethyl acetate layer was separated, washed with brine, dried ($MgSO_4$) and evaporated to an orange oil. Flash chromatography (eluant, ethyl acetate/cyclohexane, 2:1) gave **22a** as a white foam (193 mg, 74%). IR (KBr) υ_{max} 2099, 1744, 1656, 1371, 1245, 1222 cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 8.05 (1H, d, NH), 5.85 (1 H, d, H-3), 5.32 (1 H, dd, H-7), 5.21 (1 H, m, H-8), 4.45 (1 H, dd, H-9), 4.38–4.25 (2 H, m, H-4, 6), 4.12–4.00 (2 H, m, H-9, 5), 3.11 (3 H, s, COOMe), 2.0 (11 H, m, 3 x OAc, propionyl CH_2), 0.99 (3H, t, CH_3); MS 471 $(M + H)^+$, 493 $(M + Na)^+$; anal: found C 49.1; H 5.5; N 11.6. $C_{19}H_{26}N_4O_{10}$ requires C 48.5; H 5.5; N 11.9.

(4S,5R,6R)-4-Azido-5-propionylamino-6-(1R,2R,3-trihydroxypropyl)-5,6-dihydro-4H-pyran-2-carboxylic acid methyl ester ***23a***

A solution of **22a** (134 mg, 0.285 mmol) in dry methanol (3 mL) was treated with a 30% w/v solution of sodium methoxide (25μL, catalytic). The reaction was stirred under nitrogen for 2 h before removing the solvent in vacuo and chromatographing the residual gum on silica gel (eluant: 10% methanol in chloroform) to give **23a** as a white powder (55 mg, 56%). ^{1}H-NMR (DMSO-d_6): δ 8.23 (1 H, d, N-H), 5.81 (1 H, d, H-3), 4.08 (1 H, d, 7-OH), 4.01 (1 H, d, 8-OH), 4.47 (1 H, dd, H-4), 4.35 (1 H, t, 9-OH), 4.18 (1 H, d, H-6), 3.98 (1 H, m, H-5), 3.73 (3 H, s, $COOCH_3$), 3.60 (2 H, m, H-7, 8), 3.4–3.3 (2 H, m, H-9), 2.2 (2 H, q, CH_2), 1.04 (3H, t, CH_3).

(4S,5R,6R)-4-Amino-5-propionylamino-6-(1R,2R,3-trihydroxypropyl)-5,6-dihydro-4H-pyran-2-carboxylic acid trifluoroacetate ***7***

A solution of **23a** (51 mg, 0.148 mmol) in THF (3 mL) was treated with triphenylphosphine (46 mg, 1.2 equiv). After 4 h, triethylamine (1 mL) and water were added and the reaction stirred at room temperature for 3 days before partitioning between water (20 mL) and ethyl acetate (20 mL). The aqueous layer was collected and freeze-dried to give a yellow solid. The crude material was purified by preparative HPLC (2" Dynamax column C_{18}; flow rate 40 mL/min; mobile phase: A: H_2O + 0.1% trifluoroacetic acid; B: MeCN + 0.05% trifluoroacetic acid; elution gradient 0–10 min 100% A, 10–20 min gradient to 100% B, 20-25 100% B, 25-27 min gradient to 100% H_2O until 35 min) to give **7** as a white solid (35 mg, 77%). ^{1}H-NMR (D_2O): δ 5.91 (1 H, d, H-3), 4.40 (2 H, m, H-5, 6), 4.25 (1 H, m, H-4), 4.00–3.80 (2 H, m, H-8, 9), 3.7–3.6 (2 H, m, H-7, 9), 2.35 (2 H, q, CH_2), 1.12 (3 H, t, CH_3); MS 305 $(M + H)^+$; high resolution MS: found MH^+ 305.1349, calc for $C_{12}H_{21}N_2O_7$ 305.1349 (error 0.1 ppm).

Compounds **8** and **9** were prepared similarly.

(4S,5R,6R)-4-Amino-5-formylamino-6-(1R,2R,3-trihydroxypropyl)-5,6-dihydro-4H-pyran-2-carboxylic acid ***8***. ^{1}H-NMR (D_2O): δ 8.3 (1 H, s, CHO), 5.94 (1 H, d, H-3), 4.48 (2 H, m, H-4, 6), 4.3 (1 H, m, H-5), 4.0–3.8 (2 H, m, H-8, 9), 3.79–3.6 (2 H, m, H-7, 9); MS 277 $(M + H)^+$; high resolution MS: found MH^+ 277.1044, calc for $C_{10}H_{17}N_2O_7$ 277.1036 (error 3.1 ppm); HPLC: 2.81 min (98.4%).

(4S,5R,6R)-4-Amino-5-cyclopropanecarbonylamino-6-(1R,2R,3-trihydroxy-propyl)-5,6-dihydro-4H-pyran-2-carboxylic acid trifluoroacetate ***9***. IR (KBr) υ_{max} 3279, 1677, 1545, 1201, 1144 cm^{-1}. ^{1}H-NMR (D_2O): δ 5.93 (1 H, d, H-3), 4.5–4.25 (3 H, m, H-4, 5, 6), 4.0–3.85 (2 H, m, H-8, 9), 3.75–3.6 (2 H, m, H-7, 9), 1.67 (1 H, p, CH cyclopropyl), 0.92 (4 H, d, CH_2 cyclopropyl); MS 317 $(M + H)^+$; high resolution MS: found MH^+ 317.1351, calc for $C_{13}H_{21}N_2O_7$ 317.1349 (error 0.8 ppm); HPLC: 3.35 min (93%).

(4S,5R,6R)-4-Azido-5-methanesulfonylamino-6-(1S,2R,3-triacetoxy-propyl)-5,6-dihydro-4H-pyran-2-carboxylic acid methyl ester ***22d***

A solution of **21** (300 mg 0.67 mmol) in anhydrous dichloromethane (4 mL) was treated, under nitrogen, with dry pyridine (4 mL) and methanesulfonyl chloride (80 μL, 0.94 mmol, 1.4 equiv) at 0 °C. After 2 h, HPLC analysis showed that all the starting material had been consumed and a new compound formed. The organic solution was partitioned between 2 N HCl (50 mL) and ethyl acetate (50 mL). The ethyl acetate layer was separated, dried ($MgSO_4$) and evaporated to a yellow foam. Column chromatography (eluant, ethyl acetate/cyclohexane, 3:2) gave **22d** as a white foam (225 mg, 68%). ^{1}H-NMR (DMSO-d_6): δ 7.59 (d, 1 H, NH, *J* = 8 Hz), 6.04 (d, 1 H, H-3, *J* = 2 Hz), 5.42 (m, 1 H, H-7), 5.27 (m, 1 H, H-8), 4.45 (m, 1 H, H-9), 4.39 (m, 1 H, H-4), 4.25 (dd, 1 H, H-6, *J* = 2, 10 Hz), 4.09 (dd, 1 H, H-9, *J* = 8, 10 Hz), 3.45 (m, 1 H, H-5), 3.73 (s, 3 H, $COOCH_3$), 3.05 (s, 3H, NSO_2CH_3), 2.04–2.02 (m, 9 H, 3 x $OCOCH_3$). MS 510 $(M + NH_4)^+$; high resolution MS: found MH^+ 491.1079, calc for $C_{17}H_{24}N_4O_{11}S$ 491.1084 (error 0.9 ppm).

*(4S,5R,6R)-4-Azido-5-methanesulfonylamino-6-(1R,2R,3-trihydroxy-propyl)-5,6-dihydro-4H-pyran-2-carboxylic acid methyl ester **23d***

A solution of **22d** (160 mg, 0.325 mmol) in dry methanol (3 mL) was treated with a 30% w/v solution of sodium methoxide (10 μL, catalytic). The reaction was stirred under nitrogen for 17 h before removing the solvent in vacuo and chromatographing the residual gum on silica gel (eluant methanol in chloroform 1–20% gradient) to give **23d** as a white powder (85 mg, 72%): IR (KBr) υ_{max} 2101 (N_3), 1729 (C=O) cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 7.70 (br s, 1 H, NH), 5.87 (d, 1 H, H-3, J = 2 Hz), 4.70 (d, 1 H, OH, J = 3 Hz), 4.65 (d, 1 H, OH, J = 7 Hz), 4.45 (t, 1 H, 9-OH), 4.25 (dd, 1 H, H-4, J = 2, 10 Hz), 4.11 (d, 1 H, H-6, J = 10 Hz) and 3.72–3.40 (m, 5 H, H-5, H-7, H-8, H-9), 3.73 (s, 3H, $COOCH_3$), 3.06 (s, 3 H, NSO_2CH_3); MS 384 $(M + NH_4)^+$.

*(4S,5R,6R)-4-Amino-5-methanesulfonylamino-6-(1R,2R,3-trihydroxy-propyl)-5,6-dihydro-4H-pyran-2-carboxylic acid trifluoroacetate **12***

A solution of **23d** (75 mg, 0.205 mmol) in H_2O/THF 1:1 (3 mL) was treated with triphenylphosphine (107 mg, 2 equiv). After 35 h, triethylamine (150 μL) was added and the reaction stirred at room temperature for 2 days before partitioning between water (20 mL) and ethyl acetate (20 mL). The aqueous layer was collected and freeze-dried to give a yellow solid which was approximately 85% pure by HPLC analysis. The crude material was purified by preparative HPLC (conditions as for compound **7**) to give **12** as a white solid (56 mg, 85%): ^{1}H-NMR (D_2O): δ 5.78 (d, 1 H, H-3, J = 2 Hz), 4.40 (d, 1 H, H-6, J = 10 Hz), 4.19 (dd, 1 H, H-4, J = 10, 2 Hz), 4.05–3.83 (m, 4H, H-9, H-8, H-7, H-5), 3.73 (dd, 1 H, H-9, J = 12, 6 Hz), 3.23 (s, 3 H, NSO_2CH_3); MS 327 $(M + NH_4)^+$; high resolution MS: found MH^+ 327.0876, calc for $C_{10}H_{19}N_2O_8S$ 327.0862 (error 4.1 ppm); HPLC: 3.49 min (89.3%).

Compounds **13** and **14** were prepared similarly.

*(4S,5R,6R)-4-Amino-5-ethanesulfonylamino-6-(1R,2R,3- trihydroxy-propyl)-5,6-dihydro-4H-pyran-2-carboxylic acid **13**.* IR (KBr) υ_{max} 1672, 1318, 1201, 1143, 723 cm^{-1}; ^{1}H-NMR (D_2O): δ 5.9 (1 H, d, H-3), 4.4 (1 H, d, H-6), 4.22 (1 H, dd, H-4), 4.0–3.8 (4 H, m, H-5, 7, 8, 9), 3.71 (1 H, dd, H-9), 3.35 (2 H, qd, CH_2), 1.38 (3 H, t, CH_3); MS 341 $(M + H)^+$; high resolution MS: found MH^+ 341.1019, calc for $C_{11}H_{21}N_2O_8S$ 341.1019 (error 0.0 ppm).

*(4S,5R,6R)-4-Amino-5-(1,1,1-trifluoro-methanesulfonylamino) -6-(1R,2R,3-trihydroxy-propyl)-5,6-dihydro-4H-pyran-2-carboxylic acid **14**.* ^{1}H-NMR (D_2O): δ 5.9 (1 H, d, H-3), 4.53 (1 H, d, H-6), 4.35 (1 H, dd, H-4), 4.10 (1 H, t, H-5), 4.0–3.8 (4 H, m, H-7, 8, 9), 3.71 (1 H, dd, H-9); MS 381 $(M + H)^+$.

*(4S,5R,6R)-4-Guanidino-5-methanesulfonylamino-6- (1R,2R,3-trihydroxy-propyl)-5,6-dihydro-4H-pyran-2-carboxylic acid bis-(trifluoroacetate) **6***

A solution of **12** (88 mg, 0.27 mmol) in water (4 mL) was treated with potassium carbonate (75 mg, 2 equiv) to form a suspension. Over 1 h an intimate mixture of potassium carbonate (112 mg, 3 equiv) and AIMSA (100 mg, 3equiv) was added. After 24 h, a second identical mixture (AIMSA/K_2CO_3) was added over 6 h and a third portion after 48 h. The reaction was left to stir for a further 68 h before diluting with water and warming gently to give a solution. This solution was eluted through a DOWEX 50W-X8(H) ion exchange column, first with water (until eluate of pH 7 obtained) and then with 0.6 M aqueous triethylamine; 25 x 10 mL fractions were collected and evaporated. The fractions which contained a mixture of **6** and **12** by analytical HPLC were combined and freeze-dried to give a crude grey solid. This solid was subjected to preparative HPLC (conditions as described for compound **7**) affording **6** as a white solid (18 mg, 18%). ^{1}H-NMR (D_2O): δ 5.80 (d, 1H, H-3, J = 2 Hz), 4.42 (dd, 1 H, H-4, J = 10 Hz), 4.32 (d, 1 H, H-6, J = 10 Hz), 3.96–3.74 (m, 4 H, H-7, H-8, H-5, H-9), 3.67 (dd, 1 H, H-9, J = 11, 5 Hz), 3.13 (s, 3 H, NSO_2CH_3); high resolution MS: found MH^+ 369.1078, calc for $C_{11}H_{21}N_4O_8S$ 369.1080.

*(4S,5R,6R)-4-Azido-5-(4-chloro-butyrylamino)-6-(1S,2R,3-triacetoxy-propyl)-5,6-dihydro-4H-pyran-2-carboxylic acid methyl ester **24***

A solution of **21** (1.0 g, 2.22 mmol) in anhydrous dichloromethane (6 mL) was treated, under nitrogen, with di-isopropylethylamine (0.78 ml, 4.44 mmol, 2 equiv) and 4-chlorobutyryl chloride (0.4 ml, 1.6 equiv) at –50 °C. After 10 min the reaction mixture was warmed to room temperature. After 2 h the organic solution was partitioned between 2 N HCl (50 mL) and ethyl acetate (50 mL). The ethyl acetate layer was separated, washed with brine, dried ($MgSO_4$) and evaporated to a yellow oil. Column chromatography (eluant, ethyl acetate/cyclohexane, 1:1) gave **24** as a white foam (755 mg, 66%). IR (KBr) υ_{max} 3320, 2967, 2099, 1745, 1733, 1659, 1218 cm^{-1}; ^{1}H-NMR (DMSO-d_8): δ 8.2 (1 H, d, NH, J = 10 Hz), 5.88 (1 H, d, H-3, J = 2.5 Hz), 5.22 (1 H, dd, H-7, J = 2, 6 Hz), 5.22 (1 H, m, H-8), 4.43 (1 H, dd, H-9, J = 3, 12 Hz), 4.38–4.30 (2 H, m, H-4, 6), 4.11–4.00 (2 H, m, H-5, 9), 3.74 (3 H, s, $COOCH_3$), 3.66 (2 H, t, CH_2Cl), 2.20 (2 H, t, CH_2CONH), 2.0 (9 H, s, 3 x OAc), 1.93 (2 H, m, $ClCH_2CH_2$); MS 519 $(M + H)^+$, 536 $(M + NH_4)^+$; anal $C_{20}H_{27}ClN_4O_{10}$ (C, H, N).

*(4S,5R,6R)-4-Azido-5-(2-oxo-pyrrolidin-1-yl)-6-(1R,2R,3-trihydroxy-propyl)-5,6-dihydro-4H-pyran-2-carboxylic acid methyl ester **25***

A solution of **24** (750 mg, 1.45 mmol) in dry acetone (100 mL) was treated with anhydrous potassium carbonate (8.6 g) and potassium iodide (664 mg) and heated at reflux for 70 h. The resulting brown solution was filtered through Hyflo-filter aid and the pad washed thoroughly with ethyl acetate. The filtrate was evaporated in vacuo to give a brown oil. Column chromatography (eluant, ethyl acetate/cyclohexane, 1:1 to 2:1) gave the triacetate of **25** as a white foam (297 mg, 42%). Triacetate: ^{1}H-NMR (DMSO-d_6): δ 5.92 (1 H, d, H-3), 5.20 (2 H, m, H-7, 8), 4.78 (2 H, m, H-4, 6), 4.48 (1 H, dd, H-9), 4.20–4.00 (2 H, m, H-5, 9), 3.75 (3 H, s, $COOCH_3$), 3.45 (2H, m, CH_2 lactam), 2.20–1.90 (13 H, m, 3 x OAc + 4H lactam); MS 482 $(M + H)^+$.

A solution of the triacetate (290 mg, 0.602 mmol) in dry methanol (4 mL) was treated with sodium methoxide (5 mg, catalytic). The reaction was stirred under nitrogen for 2 h before removing the solvent in vacuo and chromatographing the residual gum on silica gel (eluant methanol in chloroform 1–20% gradient) to give **25** as a white powder (105 mg, 55%). ^{1}H-NMR (DMSO-d_6): δ 5.87 (1H, d, H-3), 4.94 (1H, d, 7-OH), 4.72 (1H, d, 8-OH), 4.62 (1H, d, H-6), 4.58 (1H, d, H-4), 4.16 (1H, t, 9-OH), 4.07 (1H, m, H-5), 3.76 (3H, s, $COOCH_3$), 3.63 (2H, m, NCH_2), 3.53 (1H, m, H-7), 3.46 (1H, m, H-8), 3.30 (2H, m, H-9), 2.32 (2H, m, $COCH_2$ lactam), 2.00 (2H, m, CH_2 lactam): MS 357 $(M + H)^+$, 374 $(M + NH_4)^+$.

*(4S,5R,6R)-4-Amino-5-(2-oxo-pyrrolidin-1-yl)-6-(1R,2R,3-trihydroxy-propyl)-5,6-dihydro-4H-pyran-2-carboxylic acid trifluoroacetate **11***

A solution of **25** (100 mg, 0.281 mmol) in THF (2 mL) was treated with triphenylphosphine (80 mg, 1.1 equiv). After 35 h, triethylamine (1 mL) and water (1 mL) were added and the reaction stirred at room temperature for 6 days before partitioning between water (5 mL) and ethylacetate (5 mL). The

aqueous layer was collected and freeze-dried to give a yellow solid. The crude material was purified by preparative HPLC (conditions as for compound **7**). The major peak was collected and freeze-dried to give **11** as a white solid (70 mg, 79%): IR (KBr) υ_{max} 1669, 1291, 1274, 1202, 1141, 723 cm^{-1}; ^{1}H-NMR (D_2O): δ 5.94 (1 H, d, H-3), 4.73–4.45 (3 H, m, H-4, 5, 6), 4.0–3.8 (2 H, m, H-8, 9), 3.7–3.4 (4 H, m, H-7, 8, lactam CH_2N), 2.5 (2 H, t, CH_2CO lactam), 2.15 (2 H, m, CH_2 lactam); MS 317 $(M + H)^+$; high resolution MS: found MH^+ 317.1344, calc for $C_{13}H_{21}N_2O_7$ 317.1349 (error 1.6 ppm); HPLC: 3.41 min (90%).

(4R,5R,6R)-5-(Acetyl-methyl-amino)-4-hydroxy-6-(1S,2R,3-triacetoxy-propyl)-5,6-dihydro-4H-pyran-2-carboxylic acid methyl ester ***27***

Trimethyloxonium tetrafluoroborate (10.73 g, 72.5 mmol) in anhydrous dichloromethane (400 mL) was stirred under nitrogen. Oxazoline **26** (20 g, 48.4 mmol) was then added and stirring continued at rt for 18 h. The mixture was poured into water (1 L) and 5% sodium bicarbonate solution was added. The layers were separated and the aqueous phase was further extracted with dichloromethane (2 x 500 mL). The combined organic extracts were washed with brine, dried over sodium sulfate, and the solvents were removed in vacuo to give a fawn-coloured foam (21 g). This was purified by chromatography (eluant dichloromethane/acetone, 2:1) to yield **27** as a white foam (14.9 g, 69%). A sample was crystallized from diethyl ether to give the product as fine white crystals. IR ($CHBr_3$) υ_{max} 1731, 1650, 1371, 1222 cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 6.00 (d, 1 H, H-3), 5.42 (d, 1 H, OH), 5.25 (m, 1 H, H-8), 5.19 (d, 1 H, H-7), 4.45–4.54 (m, 3 H, H-5, H-6, H-9), 4.10 (dd, 1 H, H-9), 4.04 (m, 1 H, H-4), 3.69 (s, 3 H, $COOCH_3$), 2.91 (s, 3 H, NCH_3), 2.00 (s, 3 H), 1.98 (s, 3 H), 1.94 (s, 3 H), 1.88 (s, 3 H) (3 x $OCOCH_3$ and $NCOCH_3$); MS 463 $(M + NH_4)^+$, 446 $(M + H)^+$; anal $C_{19}H_{27}NO_{11}$ (C, H, N).

(4S,5R,6R)-5-(Acetyl-methyl-amino)-4-azido-6-(1S,2R,3-triacetoxy-propyl)-5,6-dihydro-4H-pyran-2-carboxylic acid methyl ester ***28***

A stirred solution of **27** (13 g, 29.2 mmol) in anhydrous dichloromethane (130 mL) containing anhydrous pyridine (5.6 mL, 70.1 mmol), under nitrogen, was cooled to –20 °C. A solution of triflic anhydride (5.9 mL, 35 mmol) in anhydrous dichloromethane (20 mL) was added dropwise over 0.5 h. Stirring was continued for 3 h, then the solvents were removed in vacuo to give an orange foam. This was dissolved in anhydrous DMF (100 mL) and the solution was stirred under nitrogen. Sodium azide (11.4 g, 175.2 mmol) was added followed by tetrabutylammonium hydrogen sulfate (2.97 g, 8.8 mmol), and stirring was continued at rt for 18 h. The mixture was concentrated in vacuo and the residue partitioned between water (300 mL) and ethyl acetate (150 mL). The layers were separated and the aqueous phase was further extracted with ethyl acetate (2 x 100 mL). The combined organic extracts were washed with water (150 mL) and brine (50 mL), and then dried over sodium sulfate. The solvents were removed in vacuo to give an orange gum (14.4 g). This was purified by chromatography (eluant dichloromethane/acetone, 7:1) to yield **28** as an off-white foam (9.9 g, 72%). A sample was crystallized from ethyl acetate/cyclohexane. Mp 132 °C; IR (KBr) υ_{max} 2094, 1744, 1666, 1225 cm^{-1}; ^{1}H-NMR (DMSO-d_6): δ 5.91 (s, 1 H, H-3), 5.19 (m, 1 H, H-8), 5.11 (d, 1 H, H-7), 4.61–4.73 (m, 3 H, H-4, H-5, H-6), 4.43 (dd, 1 H, H-9), 4.07 (dd, 1 H, H-9), 3.72 (s, 3 H, $COOCH_3$), 2.88 (s, 3 H, NCH_3), 1.99 (s, 3 H), 1.98 (s, 3 H), 1.97 (s, 3 H), 1.91 (s, 3 H) (3 x $OCOCH_3$ and $NCOCH_3$); MS 493 $(M + NH_4)^+$, 471 $(M + H)^+$, 450 $(MNH_4 - HN_3)^+$, 428 $(MH - HN_3)^+$; anal $C_{19}H_{26}N_4O_{10}$ (C, H, N).

(4S,5R,6R)-5-(Acetyl-methyl-amino)-4-amino-6-(1R,2R,3-hydroxy-propyl)-5,6-dihydro-4H-pyran-2-carboxylic acid ***10***

To a solution of the azide **28** (5.7 g, 4.0 mmol) in anhydrous methanol (100 mL) under nitrogen, was added sodium methoxide (25 wt % solution in methanol) to pH 11. After stirring at rt for 5 h the solution was adjusted to pH 4 with Dowex-50W (H^+) resin and filtered. The filtrate was evaporated in vacuo to give a pale brown foam (4.3 g). This was purified by chromatography (eluant chloroform/methanol, 4:1) to yield the triol as a pale yellow foam (3.5 g, 85%). By ^{1}H-NMR this is a mixture of rotamers about the 5-amide in a ratio of approx. 1.6:1.

To a solution of the triol (0.1 g, 0.29 mmol) in THF (6 mL) was added triphenylphosphine (0.095 g, 0.36 mmol). After stirring at rt for 5 h, water (4.2 mL) and triethylamine (1.6 mL) were added. The resulting mixture was then stirred at 35 °C for 16 h. The THF was evaporated in vacuo and the aqueous phase was washed with ethyl acetate (2 x 10 mL). The aqueous layer was then evaporated in vacuo, and the residue purified by reverse-phase HPLC (conditions as above) to give a colourless gum after freeze-drying. This was triturated with diethyl ether (2 x 2 mL) to yield **10** as a white, amorphous powder (0.055 g, 45%). By ^{1}H-NMR this is a mixture of rotamers about the 5-amide in a ratio of approximately 3:1. IR (Nujol) υ_{max} 1675, 1629, 1461 cm^{-1}; ^{1}H-NMR (D_2O): δ 5.79 (br s, 1 H, H-3), 5.01 (m, 1 H, H-5), 4.35–4.55 (m, 2 H, H-4, H-6), 3.88 (m, 1 H, H-7), 3.79 (dd, 1 H, H-9), 3.57 (dd, 1 H, H-9), 3.42 (m, 1 H, H-8), 2.77 (s) and 2.93 (br s) (3 H, NCH_3), 2.10 (s) and 2.17 (s) (3 H, $NCOCH_3$); MS 305 $(M + H)^+$; high resolution MS: found MH^+ 305.134603, calc for $C_{12}H_{21}N_2O_7$ 305.134876.

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